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LABORATORY INVESTIGATIONS

Effects of diazepam and ketamine administered individually or in combination on regional rates of glucose utilization in rat brain

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The effects of diazepam, which acts at $GABA_A$ receptors to enhance the effects of GABA, and ketamine, a non-competitive N-methyl-D-aspartate receptor antagonist, on local rates of cerebral glucose utilization ($ICMR_{glc}$) were examined in unrestrained rats. Four groups were studied: vehicle-injected controls; and ketamine-treated, diazepam-treated and combined ketamine- and diazepam-treated animals. Ketamine alone produced a heterogeneous pattern of changes in $ICMR_{glc}$ (e.g. significant increases in the corpus callosum, olfactory tubercle and the entire Papez circuit, in addition to other limbic areas, and significant decreases in lateral habenula and some components of the auditory system). Diazepam alone statistically significantly decreased $ICMR_{glc}$ in the brain as a whole and in most areas of the cerebral cortex, thalamus and limbic system. The most remarkable effects of the two drugs administered together on $ICMR_{glc}$ occurred in the limbic system where the dramatic increases observed with ketamine alone were prevented by treatment with diazepam.

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Ketamine is a useful anaesthetic agent in certain clinical situations, such as multi-trauma or hypovolaemia, because of its unique abilities to preserve respiratory functions, arterial pressure and vascular resistance. When used alone, ketamine is accompanied by post-anaesthetic emergence reactions with vivid dreams, delirium and recurrent hallucinations.[1] These side effects can be ameliorated by the use of ketamine in combination with a benzodiazepine. The neuroanatomical pathways through which the combined actions of ketamine and benzodiazepines are mediated are not understood. Localization of the action of these drugs at the receptor level, however, has been described in detail. The site of action of ketamine is the postsynaptic excitatory N-methyl-D-aspartate (NMDA) receptor[2] which is a ligand-gated channel receptor with high Ca^{2+} permeability. Ketamine is a non-competitive inhibitor that binds to a site located in the vestibule of the ion channel of the NMDA receptor. Other compounds which block this ion channel include (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK 801), phencyclidine (PCP) and Mg^{2+}.

Benzodiazepines are used widely as anxiolytics, sedatives, hypnotics, anticonvulsants and muscle relaxants; they act by binding to a specific regulatory site on the $GABA_A$ receptor which increases the inhibitory effect of GABA.[3] The $GABA_A$ receptor is also located postsynaptically and is coupled directly to chloride channels. Distribution of receptors for both ketamine and benzodiazepines have been mapped[4,5] and show considerable overlap. As indicated by studies of regional brain energy metabolism, ketamine depresses a variety of sensory areas in the brain but activates limbic areas.[6] In contrast, benzodiazepines decrease brain energy metabolism throughout the brain.[7] The purpose of our study was to define the neuroanatomical pathways involved in the modification by diazepam of the behavioural response to ketamine. We propose that the post-anaesthetic emergence reactions to ketamine are caused by activation of the limbic system and that it is the limbic system response to ketamine that is affected by additional treatment with diazepam.

We have used the quantitative [^{14}C]deoxyglucose method[8] to study the actions of ketamine and diazepam alone

and in combination on regional rates of cerebral glucose utilization ($lCMR_{glc}$) in adult rats. Thus we sought to delineate the regions of the brain affected by each of these drugs and to examine the modifications of the responses to ketamine introduced by diazepam when the two drugs are administered together.

Materials and methods

All procedures were carried out in accordance with the National Institutes of Health Guidelines on the Care and Use of Animals and an animal study protocol approved by the National Institute of Mental Health Animal Care and Use Committee. Normal male Sprague–Dawley rats (Taconic Farms, Germantown, NY, USA) weighing 300–398 g were studied. The rats were allocated to one of four groups according to drug administration: controls (vehicle-injected) ($n=6$); diazepam 0.5 mg kg^{-1} ($n=6$); ketamine 10 mg kg^{-1} ($n=5$); and diazepam 0.25 mg kg^{-1} combined with ketamine 5 mg kg^{-1} ($n=6$). In the animals given the combination, the doses of both drugs were lower than those used in the animals given the drugs individually because it has been observed in clinical situations that lower doses of ketamine and diazepam are sufficient for anaesthesia when they are administered together. In the case of diazepam, it has been shown[7] that in rats, maximal depression of $lCMR_{glc}$ occurs at a dose of 0.3 mg kg^{-1}. It is not known if there is a difference between the effects of ketamine 5 and 10 mg kg^{-1} on $lCMR_{glc}$. Food and water were provided *ad libitum*. Rats were maintained under controlled conditions of normal humidity and temperature with alternating 12-h periods of light and darkness. Rats were prepared by insertion of polyethylene catheters into the femoral artery and both femoral veins under light halothane anaesthesia. Catheters were tunnelled under the skin and exited at the nape of the neck so that the rats could not gain access to the tubing. Rats were not restrained and could move freely throughout the experiment. At least 2 h were allowed for recovery from surgery before initiation of the experiment.

The following physiological variables were measured to evaluate the physiological state of each animal: arterial plasma glucose concentration, mean arterial pressure, arterial blood pH, P_{CO_2}, P_{O_2} and packed cell volume. Body temperature was monitored and maintained at 38°C using a YSI model 73 Tele-thermometer (Yellow Springs, OH, USA) and heat lamp.

Determination of regional rates of glucose utilization in brain

Regional rates of cerebral glucose utilization were determined using the [^{14}C]deoxyglucose method.[8] Briefly, 2–5 min after i.v. administration of ketamine and/or diazepam, rats were given an i.v. pulse of 2-deoxy-D-[1-^{14}C]glucose 50 μCi (specific activity 50–55 μCi $mmol^{-1}$; DuPont-NEN, Wilmington, DE, USA) and timed arterial blood samples were collected over the following 45 min for measurement of plasma [^{14}C]deoxyglucose and glucose concentrations. At the end of the 45-min period, rats were administered a lethal dose of pentobarbital, and their brains were removed and frozen in isopentane at –40°C. Coronal brain sections (20 μm) were cut in a cryostat maintained at –22°C and autoradiographed together with calibrated [^{14}C]methylmethacrylate standards, as described previously.[8] Rates of glucose utilization in individual brain regions and the average for the brain as a whole, weighted for the relative masses of its component parts, were determined by analysis of the autoradiograms with a computerized image processing system (MCID, Imaging Research Inc., St Catharines, Ontario, Canada) or with a Photoscan System P-1000 HS scanning densitometer (50 μm aperture) (Optronics International, Chelmsford, MA, USA). Brain regions were identified according to the rat brain atlas of Paxinos and Watson.[9] From the time courses of arterial blood [^{14}C]deoxyglucose and glucose concentrations and local tissue concentrations of ^{14}C, $lCMR_{glc}$ was calculated using the operational equation of the method.[8]

Statistical analysis

Comparisons of $lCMR_{glc}$ in each region were analysed for statistically significant differences among the four groups of rats by Bonferroni t tests for multiple comparisons[10]; results from a single region were compared across all four groups. Corrections for multiple comparisons across regions were not made in this analysis because the purpose of the study was to survey regions that were involved in individual and combined drug responses.

Results

Physiology and behaviour

Physiological variables were similar in all four groups (Table 1), but behaviour varied with treatment. Immediately after drug administration, rats treated with ketamine exhibited a cataleptic-like state of unresponsiveness for 5–10 min followed by a period of side-to-side head movements. Most animals remained fairly stationary for the remainder of the experiment. Rats in the diazepam and ketamine–diazepam groups were unconscious during the first 15–20 min of the experiment after which they began to have random movements. Control rats were alert and active for the entire experiment.

Rates of cerebral glucose utilization

In the ketamine group, $lCMR_{glc}$ increased markedly compared with controls in most regions of the limbic system, such as the entorhinal cortex, stratum lacunosum moleculare of the hippocampus, mamillary bodies, anteroventral nucleus of the thalamus, cingulate cortex, presubiculum and retrosplenial agranular cortex (Fig. 1, Table 2). There were also significant increases in $lCMR_{glc}$ in the corpus callosum

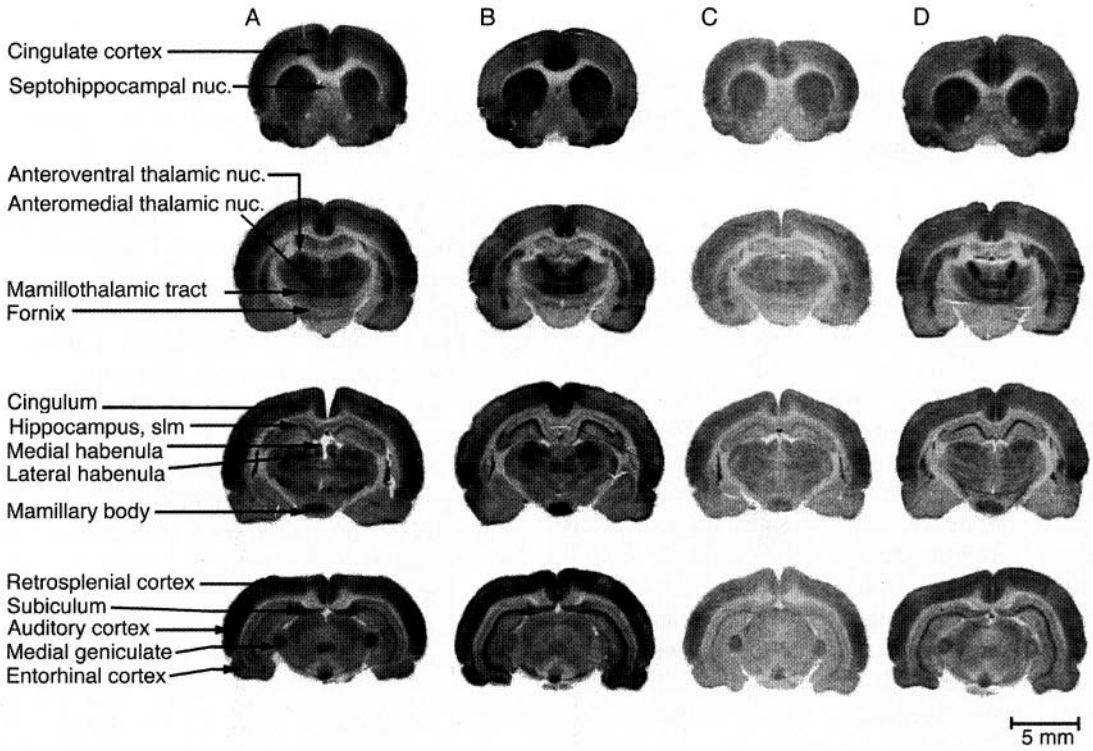

Fig 1 Representative [^{14}C]deoxyglucose autoradiograms from the brains of control (column A), ketamine-treated (column B), diazepam-treated (column C) and ketamine–diazepam-treated (column D) rats.

Table 1 Physiological variables in the control, ketamine, diazepam and ketamine–diazepam groups (mean (SEM))

	Control ($n=6$)	Ketamine ($n=5$)	Diazepam ($n=6$)	Ketamine–diazepam ($n=6$)
Body weight (g)	352 (9)	359 (10)	356 (15)	338 (8)
Arterial blood pressure (mm Hg)	108 (1)	109 (2)	104 (2)	107 (2)
Packed cell volume (%)	50 (1)	51 (1)	50 (2)	53 (1)
Arterial plasma glucose concentration (mg %)	148 (3)	142 (4)	147 (5)	155 (6)
Arterial blood pH	7.44 (0.01)	7.46 (0.01)	7.45 (0.00)	7.45 (0.04)
Arterial blood P_{CO_2} (kPa)	5.4 (0.1)	5.3 (0.1)	5.4 (0.2)	5.3 (0.1)
Arterial blood P_{O_2} (kPa)	11.0 (0.1)	10.9 (0.1)	10.9 (0.2)	10.8 (0.2)

and olfactory tubercle (Tables 2, 3). In contrast, in some of the structures of the auditory system and in the lateral habenula, $lCMR_{glc}$ statistically significantly decreased in the ketamine group. There was no change in average rate of glucose utilization in the brain as a whole as a result of the ketamine treatment (Fig. 2).

Diazepam treatment alone caused a generalized depression in $lCMR_{glc}$ (Tables 2, 3). In the brain as a whole, the rate of glucose metabolism was statistically significantly decreased by 24% compared with controls (Fig. 2) and the decreases were distributed throughout the brain, including components of the auditory, visual and sensorimotor systems. In addition, statistically significant decreases were found in many limbic areas, in supraoptic nuclei and also in the frontal and prefrontal cortex.

Treatment with the combination of ketamine and diazepam also resulted in generalized depressant effects compared with controls (Tables 2, 3). Effects in the auditory system were similar to those in the ketamine-treated rats and more profound than in the diazepam-treated rats. Most of the regions of the brain that exhibited increased metabolism with ketamine alone (i.e. limbic areas) had normal $lCMR_{glc}$ when diazepam was also administered. Effects of the combined administration of ketamine and diazepam were unique in the mamillary bodies and lateral habenula where rates of glucose utilization remained well below control rates and were similar to those found with diazepam alone.

The effects of ketamine and diazepam on glucose utilization in regions of the limbic system are illustrated in representative autoradiograms in Figure 1. The entire circuit of Papez was activated by ketamine treatment (Fig. 1, column A (control), column B (ketamine)), including the cingulate cortex, anteroventral and anteromedial thalamic nuclei, the mamillo-

Table 2 Effects of ketamine, diazepam and ketamine–diazepam treatment on $lCMR_{glc}$ (μmol 100 g^{-1} min^{-1}) in limbic and association areas, hypothalamus and fibre tracts (mean (SEM)).*$P \leq 0.05$, **$P \leq 0.01$ compared with controls (Bonferroni t tests); †$P \leq 0.05$, ††$P \leq 0.01$ compared with the diazepam group (Bonferroni t tests); ‡$P \leq 0.05$, ‡‡$P \leq 0.01$ compared with the ketamine group (Bonferroni t tests)

Region	Control (n=6)	Ketamine (n=5)	Diazepam (n=6)	Ketamine–diazepam (n=6)
Limbic system				
Fornix	58 (6)	81 (3)**	52 (3)‡‡	48 (3)‡‡
Presubiculum	95 (3)	172 (5)**	61 (6)**‡‡	97 (8)‡‡††
Cingulum	42 (2)	54 (2)**	34 (1)*‡‡	38 (3)‡‡
Retrosplenial agranular cortex	106 (6)	140 (5)**	69 (3)**‡‡	84 (5)*‡‡
Cingulate cortex	119 (4)	156 (13)*	83 (5)*‡‡	98 (7)‡‡
Anteroventral thalamic nucleus	126 (9)	183 (10)**	73 (4)**‡‡	100 (7)‡‡
Mamillary body	109 (2)	173 (7)**	44 (5)**‡‡	54 (4)**‡‡
Hippocamus, CA3, stratum lacunosum moleculare	87 (3)	137 (8)**	64 (4)‡‡	93 (8)‡‡†
Entorhinal cortex	61 (3)	78 (4)*	45 (3)*‡‡	53 (4)‡‡
Amygdala	57 (3)	75 (4)*	58 (3)‡	61 (3)
Nucleus accumbens	72 (4)	74 (9)	58 (7)	76 (9)
Interpeduncular nucleus	96 (5)	117 (7)	87 (6)‡‡	96 (4)
Medial habenula	66 (3)	63 (1)	58 (4)	57 (2)
Lateral habenula	95 (4)	76 (3)**	66 (3)**	65 (4)**
Association areas				
Prefrontal cortex	90 (3)	94 (3)	69 (3)*‡‡	78 (6)
Frontal cortex	94 (3)	95 (4)	69 (4)**‡‡	76 (4)*‡
Hypothalamus				
Paraventricular nucleus	52 (3)	50 (3)	45 (3)	42 (2)
Supraoptic nucleus	64 (3)	57 (2)	40 (3)**‡‡	41 (4)**‡‡
Myelinated fibre tracts				
Corpus callosum	37 (1)	49 (2)**	30 (2)*‡‡	30 (2)‡‡
Anterior commissure	43 (2)	50 (4)	35 (3)‡‡	36 (1)‡
Hippocampal fimbria	27 (1)	26 (1)	20 (1)**‡	21 (2)*
Cerebellar white matter	36 (2)	37 (2)	29 (2)	30 (2)

Table 3 Effects of ketamine, diazepam and ketamine–diazepam treatment on $lCMR_{glc}$ (μmol 100 g^{-1} min^{-1}) in sensory and motor brain regions (mean (SEM)).*$P \leq 0.05$, **$P \leq 0.01$ compared with controls (Bonferroni t tests); †$P \leq 0.05$, ††$P \leq 0.01$ compared with the diazepam group (Bonferroni t tests); ‡$P \leq 0.05$, ‡‡$P \leq 0.01$ compared with the ketamine group (Bonferroni t tests)

Region	Control (n=6)	Ketamine (n=5)	Diazepam (n=6)	Ketamine–diazepam (n=6)
Auditory system				
Auditory cortex	139 (5)	101 (5)**	105 (7)**	96 (6)**
Medial geniculate	115 (5)	80 (3)**	92 (6)*	75 (5)**
Inferior colliculus	173 (7)	112 (8)**	152 (10)‡	117 (3)**†
Superior olivary nucleus	119 (4)	123 (8)	134 (12)	151 (9)
Ventral cochlear nucleus	136 (7)	131 (6)	154 (13)	138 (5)
Lateral lemniscus	67 (3)	65 (2)	59 (6)	53 (4)
Visual system				
Visual cortex	93 (4)	110 (6)	85 (7)	77 (6)‡‡
Lateral geniculate	82 (3)	76 (1)	62 (4)**	68 (5)
Superior colliculus	75 (4)	81 (6)	70 (2)	63 (5)
Sensorimotor system				
Sensorimotor cortex	101 (3)	90 (5)	72 (3)**‡	72 (4)**‡
Ventrolateral thalamic nucleus	95 (5)	89 (2)	70 (5)*	76 (6)
Red nucleus	74 (1)	79 (3)	58 (3)*‡‡	61 (4)‡‡
Medial vestibular nucleus	110 (6)	102 (3)	96 (7)	98 (7)
Hypoglossal nucleus	63 (3)	56 (2)	52 (4)	55 (5)
Cerebellar grey matter	58 (1)	53 (2)	45 (4)**	47 (2)*
Olfactory system				
Olfactory tubercle	110 (5)	151 (13)**	81 (7)‡‡	100 (5)‡‡
Basal ganglia				
Caudate–putamen	111 (4)	125 (7)	84 (5)‡‡	108 (10)
Substantia nigra, pars compacta	85 (4)	102 (7)	65 (6)‡‡	86 (6)
Substantia nigra, pars reticulata	45 (5)	54 (4)	34 (3)‡	40 (3)
Globus pallidus	52 (2)	65 (4)	51 (4)	49 (5)‡

thalamic tract, mamillary bodies, fornix and subiculum. Other regions connected with this circuit, such as the retrosplenial and entorhinal cortex, dentate gyrus and molecular layer of the hippocampus, septohippocampal nucleus and the cingulum were also highly activated by ketamine treatment. Histograms of $lCMR_{glc}$ in regions of the Papez circuit following treatment with one or both of the anaesthetic agents (Fig. 3) illustrate the quantitative effects of the treatments.

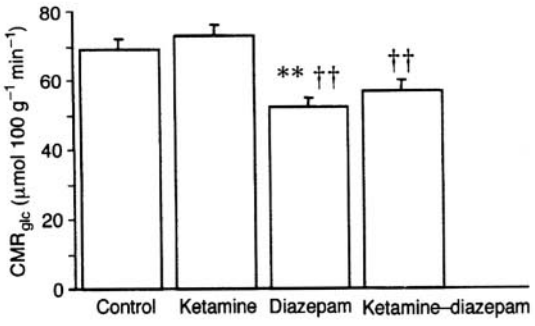

Fig 2 Rates of cerebral glucose utilization (CMR_{glc}) in the brain as a whole. Bars represent mean (SEM) obtained from six animals for each treatment, except ketamine, for which there were five animals. Statistical comparisons were made among groups by means of Bonferroni *t* tests. Statistically significant differences: $^{**}P \leq 0.01$ compared with control group; $\dagger\dagger P \leq 0.01$ compared with ketamine group.

Effects in the sensory systems are also illustrated in the autoradiograms (Fig. 1). Depression of the auditory system with ketamine treatment can be seen in the fourth row of the sections (Fig. 1, column B) in which the medial geniculate bodies and the auditory cortex are barely discernible from the surrounding tissue. This is in sharp contrast with the controls (Fig. 1, column A) in which these regions exhibit some of the highest $lCMR_{glc}$ in the brain. One other noteworthy feature of the deoxyglucose autoradiograms from the ketamine-treated rats is activation of layer V in the sensorimotor cortex (Fig. 4). The autoradiograms in the diazepam-treated rats (Fig. 1, column C) appear to have patterns of deoxyglucose distribution similar to controls, but $lCMR_{glc}$ in the entire brain appears to be depressed. Treatment with the two drugs together (Fig. 1, column D) produced patterns similar to those in the ketamine-treated rats but with a diminished response.

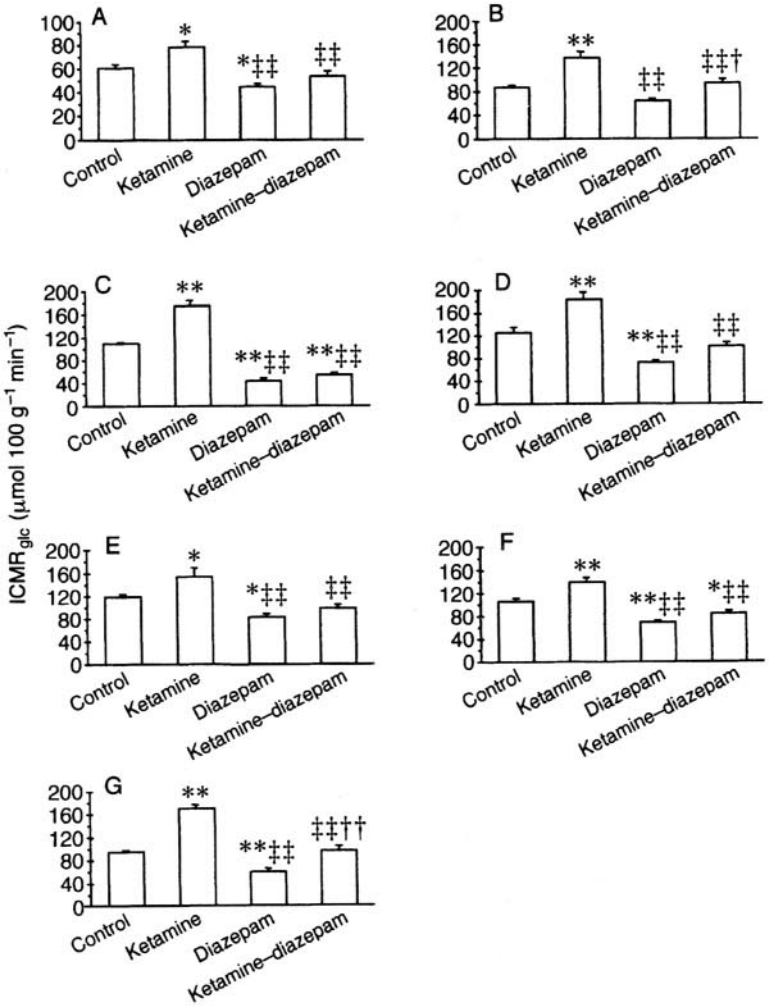

Fig 3 Local rates of cerebral glucose utilization ($lCMR_{glc}$) in the Papez circuit: A = entorhinal cortex; B = stratum lacunosum moleculare of the CA3 region of the hippocampus; C = mamillary body; D = anteroventral thalamic nucleus; E = cingulate cortex; F = retrosplenial agranular cortex; and G = presubiculum. Bars represent mean (SEM) obtained from six animals for each treatment, except ketamine for which there were five animals. Statistical comparisons were made among groups by means of Bonferroni *t* tests. Statistically significant differences: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$ compared with controls; $\dagger P \leq 0.05$, $\dagger\dagger P \leq 0.01$ compared with the diazepam group; $\ddagger P \leq 0.05$, $\ddagger\ddagger P \leq 0.01$ compared with the ketamine group.

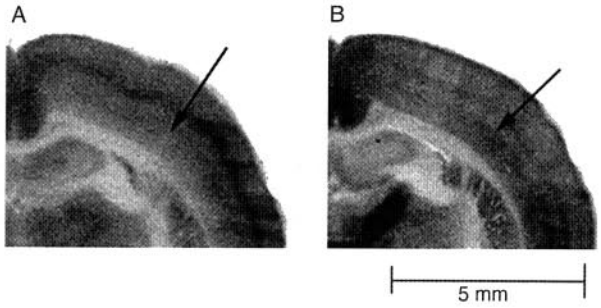

Fig 4 Enlarged views of the autoradiograms (Fig. 1) from control (A) and ketamine-treated (B) rats at the level of the sensorimotor cortex. The arrows point to cortical layer V.

Discussion

The results of this study show that some of the effects of ketamine on $lCMR_{glc}$ are reversed by additional treatment with diazepam. The reversal is specific for certain regions, particularly the limbic areas, olfactory tubercle and corpus callosum. It was in these regions that ketamine treatment alone resulted in substantially increased rates of metabolism. In brain regions in which ketamine treatment produced a decreased rate of metabolism, the combination of ketamine and diazepam produced either no change or further decreases in $lCMR_{glc}$. Treatment with diazepam alone or in combination with ketamine produced generalized decreases in cerebral energy metabolism.

The autoradiographic deoxyglucose method for determination of regional rates of glucose utilization in brain provides a powerful means of examining the functional consequences of drug administration. The approach is based on the fact that functional activity in the brain (i.e. brain work) consumes energy supplied by metabolism of glucose.[11] Areas of the brain with increased or decreased $lCMR_{glc}$ after drug administration are generally either functionally activated or depressed, respectively. Observed effects in a region are not necessarily the result of a direct drug–receptor interaction in that region but can be caused by an effect on functional activity in an entire pathway through a chain of synaptic events. Therefore, it is not a simple matter to infer from changes in $lCMR_{glc}$ alone where the primary effects of a drug occur.

In so far as regional rates of cerebral glucose utilization reflect regional functional activity,[11] our results indicate the level of function in various systems in the brain. Reductions in $lCMR_{glc}$ in the auditory system indicate decreased processing of auditory input to the brain in ketamine-treated rats. The visual system, however, was not as affected by ketamine. These data imply that at this ketamine dose, processing of auditory input is selectively affected. Depressant effects of ketamine on measures of auditory function have been reported in several species.[12, 13] In patients treated with ketamine, visual hallucinations are common but auditory hallucinations are seldom reported.

Although overall $lCMR_{glc}$ in most sensorimotor regions was unaffected by ketamine, the pattern of metabolic activity within the sensorimotor cortex was altered. In control animals, the highest rate of metabolic activity is found normally in layer IV, the site of thalamocortical input. In the ketamine-treated animals, a second deeper cortical layer can be distinguished on the autoradiograms (Fig. 3, column B). Layers V and VI receive projections via intrinsic cortical circuits and in turn project to the striatum and thalamus, respectively. This change in pattern of $lCMR_{glc}$ has been observed previously after administration of apomorphine[14] or ketamine.[15]

It has been proposed that limbic seizure activity occurs with ketamine,[16] which is consistent with the large increases in $lCMR_{glc}$ found in all of the components of the circuit of Papez,[17] including the entorhinal cortex, CA3 region of the hippocampus, presubiculum, mamillary body, anteroventral thalamic nucleus, cingulate cortex, presubiculum and retrosplenial agranular cortex. Increased $lCMR_{glc}$ in the corpus callosum produced by ketamine is consistent with increased neuronal traffic between the hemispheres.

Diazepam treatment alone resulted in widespread decreases in $lCMR_{glc}$ throughout the brain, indicating a general state of reduced neural function. This was evident in sensory systems above the brainstem level and in the limbic system, particularly in components of the Papez circuit. Quantitatively, the greatest decrease (60%) in $lCMR_{glc}$ was found in the mamillary body, which has been suggested as the site of the anti-anxiety action of benzodiazepines.[18]

In general, the effects of ketamine and diazepam in combination were not merely those of ketamine superimposed on diazepam-induced metabolic depression. The presubiculum and molecular layer of the hippocampus were the only brain regions in which $lCMR_{glc}$ increased significantly, and the inferior colliculus was the only region in which $lCMR_{glc}$ was significantly lower than that observed after treatment with diazepam alone. Brain regions in which both drugs in combination produced statistically significant decreases in $lCMR_{glc}$ compared with ketamine treatment alone included all of the components of the Papez circuit; this finding may be consistent with the clinical observation that benzodiazepines reduce the post-anaesthetic emergence reactions to ketamine while maintaining the anaesthetic effect.

The results of our study are similar to those of previous studies on the effects of ketamine on regional cerebral metabolism.[6, 19] However, our analyses, particularly of the limbic system, are much more comprehensive than those in previous reports and highlight the selectivity of ketamine for limbic areas. Previous studies of the effects of diazepam on $lCMR_{glc}$ yielded more variable results. In the study of Kelly, Ford and McCulloch,[7] in which a dose–response effect was reported, all but one of the 61 regions of the brain that were examined showed significant decreases in $lCMR_{glc}$ with diazepam 0.3 mg kg^{-1}, with the greatest per cent change (55% reduction) in the mamillary body. In our study, the effects were also greatest in the mamillary body,

but we found no effects in the amygdala, interpeduncular nucleus, globus pallidus or brainstem regions. Nehlig and colleagues[20] also found that an even higher dose of diazepam (2 mg kg^{-1}) had little or no effect in these areas. Our results do not agree with the findings of Oguchi and colleagues[19] that administration of ketamine 30 mg kg^{-1} to rats pretreated with diazepam 0.45 mg kg^{-1} had the same effects on $lCMR_{glc}$, particularly in the hippocampus, as ketamine treatment alone. The discrepancy may be a result of the use of much higher doses of ketamine and diazepam in their studies.[19]

Our results indicate that administration of diazepam together with ketamine block the 'seizure-like' effects of ketamine alone on $lCMR_{glc}$ in limbic regions while having little influence on the depressed $lCMR_{glc}$ in sensory systems. Our study does not address possible differences in the pharmacokinetics of the two drugs.

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